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Short communication

Synthesis and CNS activity of tricyclic theophylline derivatives. 8-substituted imidazo[2,1-f]theophyllines

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Abstract – Based on previously described results of pharmacological in vitro and in vivo tests of some series of pyrimido- and diazepino-[2,1-f]theophylline derivatives, N8-unsubstituted, N8-benzyl and N8-arylpiperazino-alkyl derivatives of imidazo[2,1-f]theophyllines were synthesized and tested for their CNS activity. It has been shown that tricyclic [2,1-f]theophyllines possess sedative and analgesic activity. N8-Phenylpiperazinopropyl-1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline **6** showed significant anti-serotonin and long-lasting hypothermic effects. N8-Benzyl-1,3,6,8-tetrahydroimidazo-7-on[2,1-f]theophylline **8** possess anticonvulsant properties. © 1999 Éditions scientifiques et médicales Elsevier SAS

imidazo[2,1-f]theophyllines / CNS activity of fused [2,1-f]theophyllines / arylpiperazinoalkylimidazo[2,1-f]theophyllines acting on CNS

1. Introduction

In this paper we would like to summarize the results of our investigation on chemical and pharmacological properties of derivatives in which the third heterocyclic ring is bound at position 7,8- of theophylline. In our initial studies we found that some benzyl or dialkylamino-alkyl derivatives of 1,3,6,7,8,9-hexahydropyrimido[2,1-f]theophyllines and 1,3,6,7-tetrahydro-(9H)-pyrimid-8-on[2,1-f]theophyllines (*figure 1*) changed the pharmacological profile of the mother compound (theophylline) into sedative, hypothermic, analgesic and neuroleptic-like activity [1–3].

These unexpected results seem to be related to the presence of a heterocyclic six membered ring, as well as to the basic alkylamino side chain substituted at the N9 position. Moreover, in preliminary screening it appeared that among others, the most active are the derivatives which contain arylpiperazino-propyl or its butyl homologue as an N9-substituent. This observation prompted us to synthesize the series of new compounds with seven, six and five membered rings bound at position 7,8- of theophylline and a piperazino-alkyl substituent at the N8, N9 or N10 position, to study the influence of ring size and kind of substituent in fused heterocyclic moieties on CNS activity of investigated compounds.

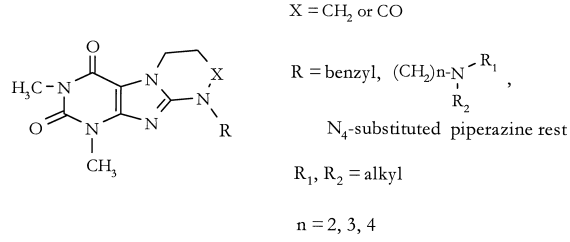

Figure 1. Structure of 9-substituted 1,3,6,7,8,9-hexahydro-(9H)-pyrimido[2,1-f]theophyllines and 1,3,6,7-tetrahydro-(9H)-pyrimid-8-on[2,1-f]theophyllines.

The enlargement of the third heterocyclic ring lead to 1,3-diazepino- and 1,3-diazepin-9-on-[2,1-f]-theophyllines [4] (*figure 2*).

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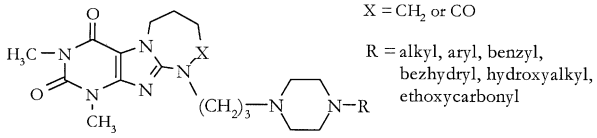

Figure 2. Structure of 10-substituted 1,3,6,7,8,9-hexahydro-(10H)-1,3-diazepino[2,1-f]theophyllines and 1,3,6,7,8,10-hexahydro-1,3-diazepin-9-on[2,1-f]theophyllines.

Both investigated groups exhibit high CNS activity. Particularly, N10-phenyl-piperazinopropyl-1,3,6,7,8,9-hexahydro-(10H)-1,3-diazepino[2,1-f]theophylline showed strong analgesic and sedative activity, but only slight hypothermic and antiamphetamine effects [4].

A similar mode of action was observed in the group of pyrimidin-8-on[2,1-f]theophyllines with a double bond between C6–C7 in the third ring [5] (*figure 3*).

Particularly, the N9-phenylpiperazinopropyl-derivate of 1,3-dihydro-(9H)-pyrimid-8-on[2,1-f]theophylline induced hypothermia and strongly decreased the spontaneous locomotor activity and amphetamine induced hyperactivity [5].

Taking into account that various 1-aryl(heteroaryl)-substituted piperazines have been reported to possess agonistic activity on serotonin receptors [6], we also investigated, in behavioral tests, the compounds with a 1-(2-pyrimido)-piperazinoalkyl substituent connected with a non-lactam tricyclic system [7] and some of their lactam analogues (**1** and **2**) (*figure 4*).

Following our research to verify the influence of the third ring in tricyclic componds on the pharmacological profile of CNS-activity, we undertook the synthesis of N8-unsubstituted (**3**) and 8-arylpiperazinoalkyl derivatives (**4–7**) of 1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline.

In order to compare CNS-activity, previously obtained N9-benzyl-1,3,6,7-tetrahydro-(9H)-pyrimid-8-on[2,1-f] theophylline [1] with its analogue in which the five member lactam ring is fused in the 7,8- position of theophylline, N8-benzyl-1,3,6,8-tetrahydroimidazol-7-on[2,1-f]theophylline **8** was synthesized.

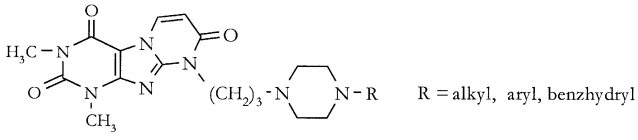

Figure 3. Structure of 9-substituted 1,3-dihydro-(9H)-pyrimidin-8-on-[2,1-f]theophyllines.

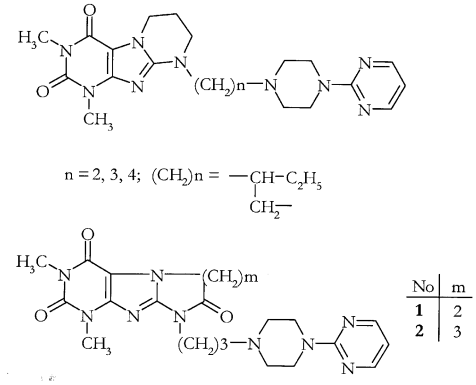

Figure 4. Structure of 1-(2-pyrimido)-piperazinoalkyl derivatives of tricyclic non-lactam and lactam system.

2. Chemistry

9-{3-[4-(2-Pyrimidynyl)-1-piperazinyl]-propyl}-1,3,6,7-tetrahydro-(9H)-pyrimid-8-on[2,1-f]theophylline (**1**) and 10-{3-[4-(2-pyrimidynyl)-1-piperazinyl]-propyl}-1,3,6,7,8,10-hexahydro-1,3-diazepin-9-on[2,1-f]theophylline (**2**) were prepared according to the procedures described previously [6].

In the reaction of 7-β-bromoethyl-8-bromotheophylline [8] with ammonia 8-unsubstituted 1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline, (**3**) was obtained. Arylpiperazino-alkyl derivatives of 1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline (**4–7**) were obtained in a several step synthesis (*figure 5*).

8-β-Hydroxyethyl-1,3,6,7-tetrahydro-(8H)-imidazo-[2,1-f]theophylline (**I**) and its homologue (**Ia**) were prepared by condensation of 7-β-bromoethyl-8-bromotheophylline with 2-aminoethanol or 3-aminopropan-1-ol. Compounds **I** and **Ia**, upon bromination with PBr_3 in $CHCl_3$, yielded **II** and **IIa**. Aminolysis of compound **II** and **IIa** with a double amount of phenylpiperazine or 2′-pyrimidyl-piperazine in anhydrous toluene in the presence of K_2CO_3 gave the compounds **4–7**.

N8-Benzyl-1,3,6,8-tetrahydroimidazol-7-on[2,1-f]theophylline **8** was synthesized via cyclization of 8-benzyl-aminotheophylline-7-acetic acid [9] in boiling acetic anhydride (*figure 6*).

3. Pharmacological results and discussion

The compounds **1–8** were evaluated for their influence on the CNS. Their acute toxicity assessed in mice as the LD_{50} (confidence limits) was as follows:

Compound **1**: 235 mg/kg (178–310)

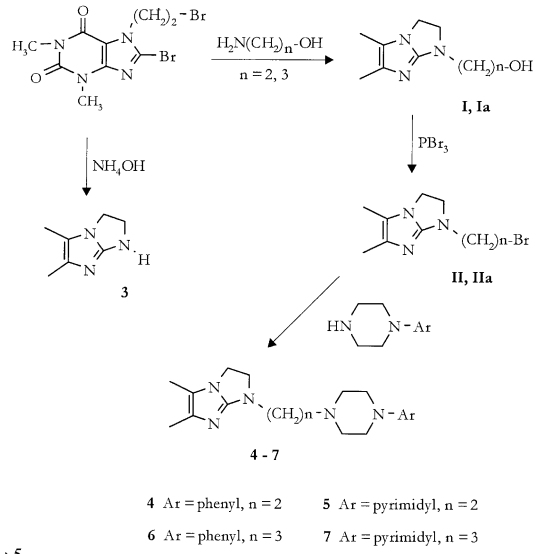

Figure 5. Arylpiperazino-alkyl derivatives of 1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline (**4**–**7**).

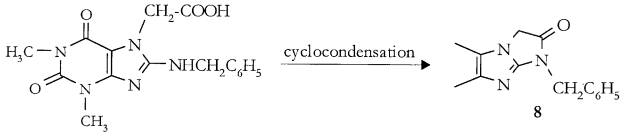

Figure 6. N8-Benzyl-1,3,6,8-tetrahydroimidazol-7-on[2,1-f] theophylline **8**.

Table I. The influence of the compounds (0.1 LD_{50}) on the head twitch responses of mice receiving L-5HTP (180 mg/kg i.p.).

Compound	Mean ± SE number of head twitch responses
Tylose	11.4 ± 2.9
1	18.0 ± 4.3
2	14.5 ± 4.0
3	15.0 ± 4.2
4	3.6* ± 1.3
5	12.3 ± 5.5
6	1.8** ± 0.7
7	9.3 ± 3.2
8	12.7 ± 3.7

The compounds were injected 60 min before L-5HTP.
* $P < 0.05$, ** $P < 0.01$ (Student's *t*-test).

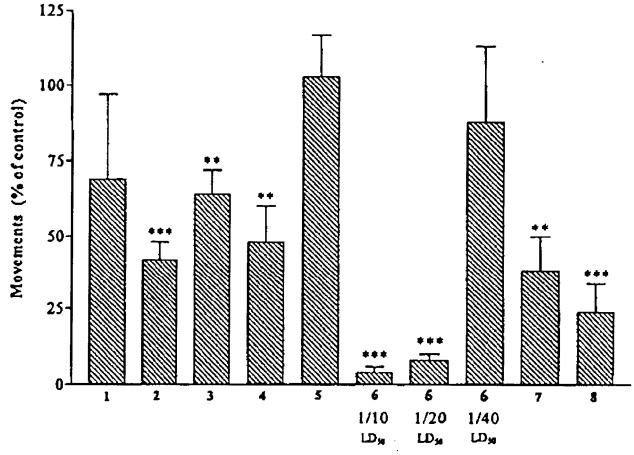

Figure 7. The influence of compounds (0.1 LD_{50}) on spontaneous locomotor activity of mice.

Compound **2**: 560 mg/kg (478–655)
Compound **3**: 320 mg/kg (269–381)
Compound **4**: 175 mg/kg (117–263)
Compound **5**: 320 mg/kg (264–387)
Compound **6**: 540 mg/kg (439–664)
Compound **7**: 2 000 mg/kg
Compound **8**: 2 000 mg/kg

The CNS effects of all these substances (1/10 LD_{50}) were studied in several tests, and it was observed that substances **2**, **3**, **4**, **6**, **7** and **8** induced marked sedative effects in the locomotor activity test (*figure 7*).

Compound **6** had the strongest action, which reduced the activity of mice by about 90% (1/10 and 1/20 LD_{50}). These doses of substance **6** also antagonized amphetamine-induced hyperactivity in mice, but did not change apomorphine-induced stereotypy or haloperidol-induced catalepsy in rats. Compounds **4** and **6** also had antiserotonin effects, as they diminished 5HTP-induced head-twitch reactions of mice (*table I*). Hypothermic action was observed after the administration of compounds **1**, **6**, **7** and **8** (*figure 8*). The most apparent and long-lasting effects were observed for compounds **6** and **8**. Analgesic action in the hot plate test was induced in mice by nearly all substances, except for compound **8** (*table II*).

These effects were of rather a short duration (up to 90 min after injection). Compound **8** had anticonvulsant action in the pentetrazole test in mice, as it prevented the tonic phase of the seizures and lethality of the animals. Reserpine-induced hypothermia was slightly reversed by substances **1**, **3** and **4**, and this action suggests some antidepressant properties of these three substances. How-

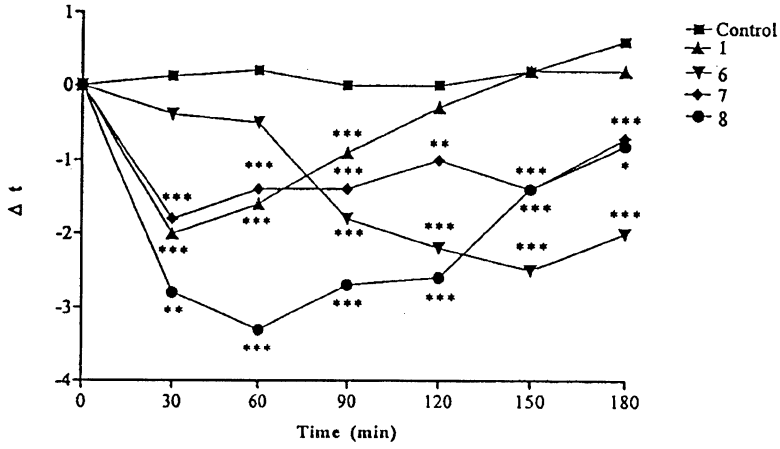

Figure 8. The influence of compounds (0.1 LD_{50}) on body temperature of normothermic mice.

ever, we observed neurotoxic effects in the rota-rod test in mice following injection of nearly all compounds tested.

4. Conclusion

Analysing the results of our preliminary investigations on chemical and pharmacological properties of tricyclic theophylline derivatives from the point of view of structure-activity relationships, it may be concluded that:

1. In contrast to mother theophylline, all investigated tricyclic compounds generally exhibited a strong sedative action, particularly those which contained the basic aminoalkyl side chain, substituted on the third bound ring at N8 of imidazole, N9 of pyrimidine and N10 of diazepine. Their sedative properties were confirmed in a number of behavioral tests (in mice).

2. The replacement of the methylene group with a carbonyl one in pyrimido[2,1-f]theophyllines or diazepino[2,1-f]theophyllines (lactam structure) did not have a significant influence on the direction of pharmacological activity.

3. The potency of sedative action depends significantly on the kind of N-substituted basic side chain. Variation of the alkylamino substituents confirmed unequivocally that the most apparent CNS activity was exerted by the compounds with phenylpiperazinopropyl- or phenylpiperazinobutyl- substituents.

4. Length reduction of the alkylene chain between dialkylamino substituents and tricyclic substituents from three or four to two carbon atoms resulted mostly in an increase of toxicity.

5. The replacement of the phenylpiperazinoalkyl substituent with an (ω-4-pyrimidinyl)-piperazino-alkyl side chain generally decreased the activity and revealed some neurotoxic effects.

Table II. The influence of the tested compounds on the reactivity of mice in the hot plate test.

Compound 0.1 LD_{50}	Reaction time (s) of mice after injection (min)			
	30	60	90	120
Vehicle	8.9 ± 0.6	9.3 ± 0.6	9.5 ± 0.7	9.1 ± 0.7
1	14.5*** ± 0.9	13.4** ± 0.8	15.1** ± 1.2	11.5 ± 0.9
2	13.0*** ± 0.7	11.7 ± 0.9	10.8 ± 0.6	10.4 ± 0.5
3	10.8 ± 0.6	13.8** ± 1.1	10.4 ± 0.6	10.8 ± 0.7
4	14.7*** ± 1.0	12.3* ± 0.8	13.8* ± 1.1	13.4* ± 1.2
5	13.4** ± 1.1	12.9* ± 1.0	11.2 ± 0.7	11.2 ± 0.8
6	13.0** ± 1.0	14.3** ± 1.2	12.3 ± 1.2	11.5 ± 0.9
7	14.7*** ± 1.1	13.2*** ± 1.0	10.6 ± 0.6	11.2 ± 0.8
8	10.1 ± 0.6	10.9 ± 0.7	9.3 ± 0.5	10.2 ± 0.6

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$ (Student's *t*-test).

6. All phenylpiperazinoalkyl substituted tricyclic [2,1-f]theophyllines reduced the amphetamine induced hyperactivity.

Pyrimido[2,1-f]theophyllines with phenylpiperazinoalkyl side chains showed mainly analgesic and neuroleptic-like properties. The pyrimidinyl-piperazino analogues exhibited agonistic activity on 5-HT_{1A} receptors. Their diazepino homologues exerted apparent analgesic activity.

7. Some of previously described tricyclic theophylline derivatives, evaluated for the affinities for 5-HT receptors, were classified as postsynaptic antagonists or partial agonists of 5-HT_{1A} receptors [6].

Pyrimido[2,1-f-]theophyllines were also tested for affinity to adenosine receptors [10]. They were classified as adenosine receptor subtype A_1 antagonists.

Some of the presented data on tricyclic [2,1-f-]theophyllines summarized in this article remains open to further investigation.

5. Experimental protocol

5.1. Chemistry

Melting points were determined on a Boetius apparatus and are uncorrected. UV spectra were obtained on the UV-VIS Lambda 12 spectrophotometer (Perkin Elmer) (conc. 5×10^{-5} M/dm^3 in methanol). IR spectra were determined on the Specord M 80 spectrophotometer (Carl Zeiss Jena) in KBr pellets. ^{1}H-NMR spectra were obtained on a Brucker AC200 F spectrometer in $CDCl_3$ (except **I** measured in DMSO) solution using TMS as internal standard. Chemical shifts are reported in δ units. Coupling constants are reported in Herz. Elemental analyses indicated by the symbols were within ± 0.4% of the theoretical value. TLC was performed on Merck plates (Kieselgel 60 F_{254} with S = benzene:acetone: methanol (1:1:1) + 2 drops NH_4OH.

5.1.1. 8-β-Hydroxyethyl-1,3,6,7-tetrahydro-(8H)-imidazo-[2,1-f]theophylline **I**

A mixture of 7-β-bromoethyl-8-bromotheophylline (5.0 g; 0.014 mol), 2-aminoethanol (5 cm^3; 0.08 mol) and 40 cm^3 of ethanol was refluxed for 5 h. After refrigeration the product was filtered off and recrystallized. Yield 69%; m.p. 238–240 °C (ethanol). R_f 0.74, UV λ_{max} nm 299.4 (log ε 4.30). ^{1}H-NMR 3.17 (s, 3H, N3CH_3), 3.34 (s, 3H, N1CH_3), 3.30–3.36 (m, 2H, $\underline{CH_2}OH$), 3.57–3.64 (t, 2H, N8CH_2), 3.91–3.98 (t, 2H, $\overline{C7H_2}$), 4.05–4.12 (t, 2H, C6H_2), 4.82–4.86 (t, 1H, OH). IR (cm^{-1}) 3 300 (OH), 2 800–2 950 $(CH_2)_n$, 1 650 (CO), 1 450 (CH_2–N). Anal. $C_{11}H_{15}O_3N_5$; C, H, N.

5.1.2. 8-γ-Hydroxypropyl-1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline **Ia**

The title compound was obtained similarly to compound **I** using 3-amino-1-propanol and isobutanol as a solvent. The mixture was refluxed for 10 h. After refrigeration, the separated product was recrystallized. Yield 54%; m.p. 186–188 °C (isopropanol). R_f 0.74, UV λ_{max} nm 298.5 (log ε 4.19). ^{1}H-NMR 1.80–1.90 (m, 2H, $CH_2\underline{CH_2}CH_2$) 3.34 (s, 3H, N3CH_3), 3.48 (s, 3H, N1CH_3), 3.49–3.55 (m, 2H, $\underline{CH_2}OH$), 3.66–3.71 (t, 2H, N8CH_2), 3.92–3.98 (t, 2H, $\overline{C7H_2}$), 4.22–4.28 (t, 2H, C6H_2). IR (cm^{-1}) 3 300–3 450 (OH), 2 800–2 950 $(CH_2)_n$, 1 650 (CO), 1 450 (CH_2–N). Anal. $C_{12}H_{17}O_3N_5$; C, H, N.

5.1.3. 8-(β-Bromoethyl)-1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline **II** *and 1,3-8-(γ-bromopropyl)-1,3,6,7-tetrahydro-(8H)-imidazo[2,1-f]theophylline* **IIa**

These two compounds were obtained similarly. To 0.01 mol of compound **I** or **Ia**, fourfold amounts of PBr_3 in 25 cm^3 of $CHCl_3$ was added and refluxed for 5 h. Then the solvent and the excess of PBr_3 were distilled off under reduced pressure and to the residue, water was added. The mixture was alkalized with 10% NaOH (to pH 8). The crude products were separated and recrystallized.

Compound **II**. Yield 92%; m.p. 210–212 °C (ethanol). R_f 0.87, UV λ_{max} nm 298.5 (log ε 4.20). ^{1}H-NMR 3.36 (s, 3H, N3CH_3), 3.51 (s, 3H, N1CH_3), 3.60–3.65 (t, 2H, CH_2Br), 3.76–3.81 (t, 2H, N8CH_2), 4.02–4.08 (t, 2H, C7H_2), 4.24–4.31 (t, 2H, C6H_2). IR (cm^{-1}) 2 800–2 950 $(CH_2)_n$, 1 650 (CO), 1 450 (CH_2–N). Anal. $C_{11}H_{14}O_2N_5Br$; C, H, N.

Compound **IIa**. Yield 94%; m.p. 162–164 °C (50° ethanol). R_f 0.87, UV λ_{max} nm 299.4 (log ε 4.11). ^{1}H-NMR 2.21–2.32 (m, 2H, $CH_2\underline{CH_2}CH_2$) 3.37 (s, 3H, N3CH_3), 3.52 (s, 3H, N1CH_3), 3.47–3.54 (t, 2H, CH_2Br), 3.88–3.95 (t, 2H, C7H_2), 4.05–4.12 (t, 2H, C6H_2). IR (cm^{-1}) 2 800–2 950 $(CH_2)_n$, 1 650 (CO), 1 450 (CH_2–N). Anal. $C_{12}H_{16}O_2N_5Br$; C, H, N.

5.1.4. 1,3,6,7-Tetrahydro-(8H)-imidazo[2,1-f]theophylline **3**

A mixture of 7-β-bromoethyl-8-bromotheophylline (4.0 g; 0.011 mol), 5 cm^3 of 25% ammonia and 15 cm^3 of ethanol was heated in an autoclave at 180–190 °C for 5 h. Then the solution was evaporated and the residue was refluxed with isopropanol to separate the inorganic salts. The hot mixture was filtered off and the filtrate was distilled off under reduced pressure. The crude product was recrystallized from ethanol; m.p. 270–272 °C, yield 60%. Compound **3** was obtained by Eckstein et al. [11] using another method.

5.1.5. General procedure for compounds **4–7**

Compounds **4–7** were obtained by aminolysis of compounds **II** or **IIa** with double the amount of phenylpiperazine or 2′-pyrimidynyl-piperazine. The reaction was carried out in anhydrous toluene in the presence of a stoichiometric amount of K_2CO_3 and refluxed for 5 h. The inorganic salt was filtered off from the hot mixture and the filtrate was refrigerated. The crystalline products were filtered off and recrystallized.

5.1.5.1. 8-{2-[4-(Phenyl)-1-piperazinyl]-ethyl}-1,3,6,7,-tetrahydro-(8H)-imidazo[2,1-f]theophylline **4**

Yield 52%; m.p. 164–166 °C (ethanol + butyl acetate). R_f 0.80, UV λ_{max} nm 244.0 (log ε 4.09), 296.7 (log ε 4.23). ^{1}H-NMR 2.70–2.75 (m, 10H, $CH_2N(CH_2)_4N$), 3.19 (t, J = 4.8, 2H, N8CH_2), 3.37 (s, 3H, N3CH_3), 3.51 (S, 3H, N1CH_3), 3.99 (t, J = 7.7, 2H, C7H_2), 4.22 (t, J = 7.8, 2H, C6CH_2), 6.86–6.93 (m, 3H, 3′,4′,5′-phenyl), 7.26 (m, 2H, 2′,6′-phenyl). Anal. $C_{21}H_{27}O_2N_7$, C, H, N.

5.1.5.2. 8-{2-[4-(2-Pyrimidynyl)-1-piperazinyl]-ethyl}-1,3,6,7,-tetrahydro-(8H)-imidazo[2,1-f]theophylline **5**

Yield 91%; m.p. 194–195 °C (toluene). R_f 0.50, UV λ_{max} nm 242.0 (log ε 4.14), 301.0 (log ε 3.99). ^{1}H-NMR 2.55–2.70 (m, 10H, $CH_2N(CH_2)_4N$), 3.37 (s, 3H, N3CH_3), 3.51 (s, 3H, N1CH_3), 3.78 (t, J = 5.0, 2H, N8CH_2), 3.35–4.03 (m, 2H, C7H_2), 4.18–4.26 (m, 2H, C6CH_2), 6.48 (m, J = 4.7, 1H, 5′-pyrimidyl), 8.30 (d, J = 4.8, 2H, 4′,6′-pyrimidyl). Anal. $C_{19}H_{25}O_2N_9$, C, H, N.

5.1.5.3. 8-{2-[4-(Phenyl)-1-piperazinyl]-propyl}-1,3,6,7,-tetrahydro-(8H)-imidazo[2,1-f]theophylline **6**

Yield 79%; m.p. 192–194 °C (toluene). R_f 0.75, UV λ_{max} nm 246.0 (log ε 4.09), 299.0 (log ε 4.09). ^{1}H-NMR 1.84–1.92 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.44–2.63 (m, 10H, $CH_2N(CH_2)_4N$), 3.19 (t, J = 4.7, 2H, N8CH_2), 3.35 (s, 3H, N3CH_3), 3.47 (s, 3H, N1CH_3), 3.86 (t, J = 8.0, 2H, C7H_2), 4.20 (t, J = 8.0, 2H, C6CH_2), 6.84–6.93 (m, 3H, 3′,4′,5′-phenyl), 7.24 (t, J = 7.8, 2H, 2′,6′-phenyl). Anal. $C_{22}H_{29}O_2N_7$, C, H, N.

5.1.5.4. 8-{2-[4-(2-Pyrimidynyl)-1-piperazinyl]-propyl}-1,3,6,7,-tetrahydro-(8H)-imidazo[2,1-f]theophylline **7**

Yield 79%; m.p. 192–194 °C (toluene). R_f 0.59, UV λ_{max} nm 242.0 (log ε 4.15), 301.0 (log ε 3.94). ^{1}H-NMR 1.84–1.92 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.43–2.53 (m, 10H, $CH_2N(CH_2)_4N$), 3.35 (s, 3H, N3CH_3), 3.41 (t, J = 4.7, 2H, N8CH_2), 3.49 (s, 3H, N1CH_3), 3.75–3.92 (m, 2H, C7H_2), 4.21 (t, J = 8.0, 2H, C6CH_2), 6.47 (t, J = 4.8, 1H, 5′-pyrimidyl), 8.30 (d, J = 4.8, 2H, 4′,6′-pyrimidyl). Anal. $C_{20}H_{27}O_2N_9$, C, H, N.

5.1.5.5. 8-Benzyl-1,3,6,8-tetrahydroimidazol-7-on[2,1-f]theophylline **8**

A mixture of 8-benzylaminotheophyllino-7-acetic acid (3.43 g; 0.01 mol) and 15 cm^3 of acetic anhydride was refluxed for 3 h. After cooling, the product was separated by filtration, washed with a small amount of methanol and recrystallized. Yield 50%, m.p. 216–218 °C (methanol). R_f 0.92 (chloroform:methanol: 25% NH_4OH (8:2:0.25)), UV λ_{max} nm 300.0 (log ε 4.01) (conc. 5×10^{-5} M/dm^3 in chloroform). ^{1}H-NMR 3.33 (s, 3H, N3CH_3), 3.58 (s, 3H, N1CH_3), 4.71 (s, 2H, N8$\underline{CH_2}C_6H_5$), 4.93 (s, 2H, C6CH_2), 7.33–7.48 (m, 5H, C_6H_5). Anal. $C_{16}H_{15}O_3N_5$, C, H, N.

5.2. Pharmacology

The experiments were performed on male Swiss mice (19–26 g), and on Wistar rats (180–200 g), kept at ambient temperature on a 12 h light/dark schedule, with free access to food and water before the experiments. The experimental groups consisted of 5–8 animals each. All investigated compounds were injected i.p., 1 h before the test, as a suspension in 0.5% tylose solution.

The doses corresponding to 0.1 LD_{50} of the compounds were administered in all tests. The acute toxicity of the compounds was assessed in mice as the LD_{50} calculated on the basis of mortality within 48 h [12].

Spontaneous locomotor activity and amphetamine-induced hyperactivity in mice were observed in a photoresistor actometer for 30 min. Analgesic effects were assessed in the hot plate (56 °C) test in mice, and body temperature was measured with a thermistor thermometer in the rectum of mice. Antipsychotic action was evaluated in the tests of amphetamine (5 mg/kg s.c.)-induced hyperactivity of mice and apomorphine (3 mg/kg s.c.)-induced stereotypy in rats. Antiparkinsonian effects were measured by investigation of the influence of the compounds on haloperidol (1 mg/kg)-induced catalepsy in rats. Anticonvulsant activity was assessed in pentetrazole (90 mg/kg s.c.)-induced seizures in mice. The antidepressant action was evaluated using the test of reserpine-induced hypothermia in mice (reserpine 2.5 mg/kg s.c., 20 h before injection of the investigated substances, and then body temperature was measured at 30 min intervals for 3 h). The influence of the compounds on serotonin neurotransmission was evaluated in the head-twitch responses of mice following 5HTP injection [13, 14]. Neurotoxic properties were observed in the rota-rod test [15, 16] in mice.

Statistical analysis was carried out using the Student's *t*-test (convulsions) and Mann-Whitney U test (catalepsy, stereotypy).

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